201-15838

NCIC OPPT/DC/USEPA/US
Sent by: JuanB Perez

03/21/2005 08:08 AM

To NCIC HPV@EPA
cc
bcc
Subject Re: HPV Submission CASNO 27090-63-7

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US Environmental Protection Agency
Office of Pollution Prevention and Toxics Docket
Non-Confidential Information Center (MC 7407T)
(operated by ASRC Aerospace Corporation)
1301 Constitution Ave NW Room B146 EPA West
Washington DC 20460
phone 202-566-0280 * fax 202-566-0282 * e-mail oppt.ncic@epa.gov

EJ Rauckman <erauckman@charter.net>

EJ Rauckman
<erauckman@charternet>
03/18/2005 03:51 PM

To NCIC OPPT@EPA, Rtk Chem@EPA
cc
Subject HPV Submission CASNO 27090-63-7

HPV Coordinator,

On behalf of Solutia, I am submitting the test plan and robust summaries for the HPV substance:

Tetrabutylhexamethylenediamine (TBHMD), CAS no. 27090-63-7

These documents are in PDF format (unlocked). If you have any questions or require the documents in another format please contact me by email or telephone.

Best regards,

Elmer Rauckman, PhD, DABT (for Solutia)

Toxicology and Regulatory Affairs

Freeburg IL 62243

rauckman@toxicsolutions.com

618-539-5280

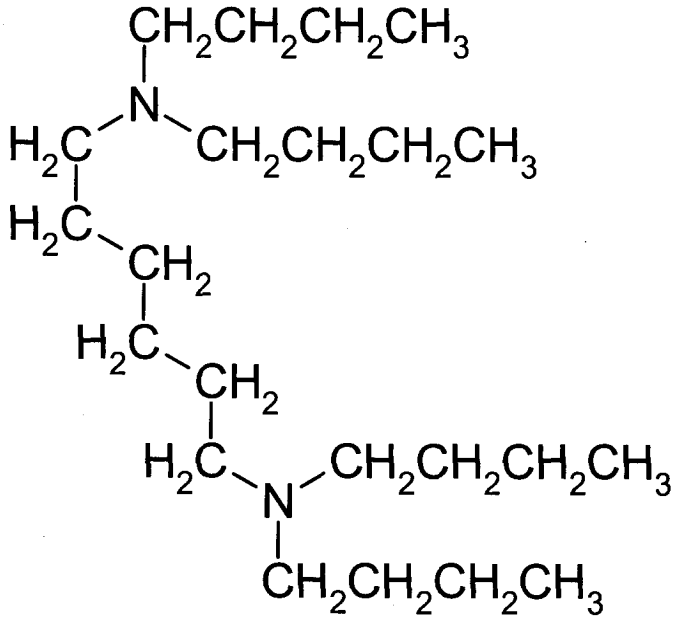

TBHMD Test Plan.pdf


TBHMD Rob Sum.pdf

201-15838

Tetrabutylhexamethylenediamine

CAS Number 27090-63-7



U.S. EPA HPV Challenge Program Submission

Submitted by:

Solutia

Prepared by:
Toxicology and Regulatory Affairs
1201 Anise Court
Freeburg IL 62243
618-539-5280

December 30, 2004

Table of Contents

Executive Overview

Tetrabutylhexamethylenediamine (TBHMD), CAS no. 27090-63-7, is an aliphatic tertiary diamine that is an intermediate in the manufacture of 1,6-bis(dibutylethylammonium)hexane hydroxide (BQAOH). BQAOH is used as a process aid in the manufacture of adiponitrile, which is a intermediate in Solutia's manufacturing process for nylon-6,6. A portion of the TBHMD is sold commercially and used for the same purpose. No other current commercial uses are known for this material. Solutia produces TBHMD at only one site and, except for the portion sold commercially, all TBHMD manufactured by Solutia is converted to BQAOH.

TBHMD is a clear oily liquid with an acrid odor. It has low volatility (estimated boiling point at 1013 hPa of 380°C and vapor pressure less than 0.015 hPa @ 25˚C) and is relatively insoluble in water (water solubility 120 mg/L). Because it is an amine, its solubility is pH dependent with greater solubility at lower (more acidic) values of pH.

In the environment, based on physicochemical properties, TBHMD has potential to bioaccumulate (Log $K_{o/w}$ > 3) and will distribute primarily to water and sediment where it will be subject to limited volatilization and assumed biodegradation under conditions favorable to bacteria. It is stable to hydrolysis but expected to react rapidly with atmospheric hydroxyl radicals with a half-life of less than an hour. Toxicity to aquatic species is unknown.

The acute oral toxicity of TBHMD is moderate with an LD_{50} value of 380 mg/kg reported in a rat gavage studies. The dermal LD_{50} in rabbits was found to be between 398 and 631 mg/kg and the material is corrosive to the skin. No inhalation data are available. Based on worker experience, the material is considered a skin sensitizer in humans.

A subchronic gavage study revealed that TBHMD is hepatotoxic in rats with a NOAEL of 2 mg/kg-day and a LOAEL (slight effects in females only) of 5 mg/kg-day. No other specific target organs were identified in this study.

Genetic toxicity data for TBHMD are not available. Although genotoxicity data are available for hexamethylenediamine and tributyl amine showing lack of genetic toxicity, neither is considered an adequate analog for TBHMD. Genotoxicity studies appropriate to the U.S. EPA-HPV Program are proposed.

Neither the reproductive nor the developmental toxicity of TBHMD has been studied experimentally. A reproductive and developmental toxicity screening study to investigate the potential of this material to affect reproductive and developmental parameters is proposed.

It is concluded that the available information for TBHMD adequately fills some of the HPV Program data elements. Studies have been proposed to investigate the biodegradation, aquatic toxicity, genotoxicity and reproductive/developmental toxicity of TBHMD.

Testing Plan and Rationale

Testing Plan in Tabular Format

CAS No. 27090-63-7 TBHMD	Information Available?	OECD Study ?	GLP Study?	Supporting Information?	Estimation Method?	Acceptable?	Testing Recommended?	
HPV Endpoint								
Physical Chemical								
Melting Point	Y	N	N	N	N	N	N	
Boiling Point	Y	N	N	Y	Y	Y	N	
Vapor Pressure	Y	N	N	N	Y	Y	N	
Partition Coefficient	Y	N	N	N	Y	Y	N	
Water Solubility	Y	N	N	Y	N	Y	N	
Environmental & Fate								
Photo-Degradation	Y	N	N	N	Y	Y	N	
Water Stability	Y	N	N	Y	Y	Y	N	
Transport	Y	N	N	N	Y	Y	N	
Biodegradation	N	N	N	Y	N	N	Y	
Ecotoxicity								
Acute Fish	Y	N	N	N	Y	N	Y	
Acute Invertebrate	Y	N	N	N	Y	N	Y	
Acute Algae	Y	N	N	N	Y	N	Y	
Toxicity								
Acute	Y	N	N	N	N	Y	N	
Repeated Dose	Y	N	Y	Y	N	Y	N	
Genetic Toxicology *"in vitro"*	N	N	N	N	N	N	Y	
Genetic Toxicology "*in vivo*"	N	N	N	N	N	N	Y	
Reproductive	N	N	N	N	N	N	Y	
Developmental	N	N	N	N	N	N	Y	

Introduction

(Tetrabutylhexamethylenediamine (TBHMD), CAS no. 27090-63-7, is an aliphatic tertiary diamine that is used as an intermediate in the manufacture of 1,6-bis(dibutylethylammonium)hexane hydroxide (BQAOH). BQAOH is used as a process aid in the manufacture of adiponitrile, which is primarily used as a intermediate in Solutia's manufacturing process for nylon-6,6. A portion of the TBHMD produced is sold commercially and used for the same purpose. No other current commercial uses are known for TBHMD.

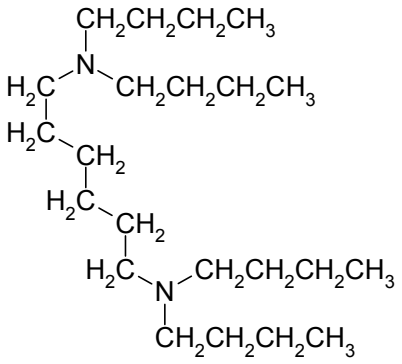
TBHMD is a clear oily liquid with an acrid odor. This material has low volatility (estimated boiling point at 1013 hPa of 380°C and vapor pressure less than 0.015 hPa @ 25˚C) and is relatively insoluble in pure water (water solubility 120 mg/L). Because it is an amine, its solubility is pH dependent with greater solubility at lower (more acidic) values of pH.

This material is batch-produced in a single reactor on a daily basis. Only the few workers converting hexamethylene diamine to BQAOH are potentially exposed to TBHMD as it serves only as an intermediate. Worker exposure is minimized by the use of closed systems and mandated personal protective equipment. Handling of TBHMD is restricted to trained personnel using personal protective equipment appropriate for handling a skin-corrosive liquid. Based on worker experience, the material is also considered a human skin sensitizer and appropriate procedures and equipment for handling a sensitizer are employed. Because of its acrid odor, the material possesses good warning properties. There is no established OSHA PEL or ACGIH TLV for TBHMD.

TBHMD is also known as (1):

- 1,6-Hexanediamine, N,N,N',N'-tetrabutyl- (8CI 9CI)
- N,N,N',N'-Tetrabutylhexamethylenediamine

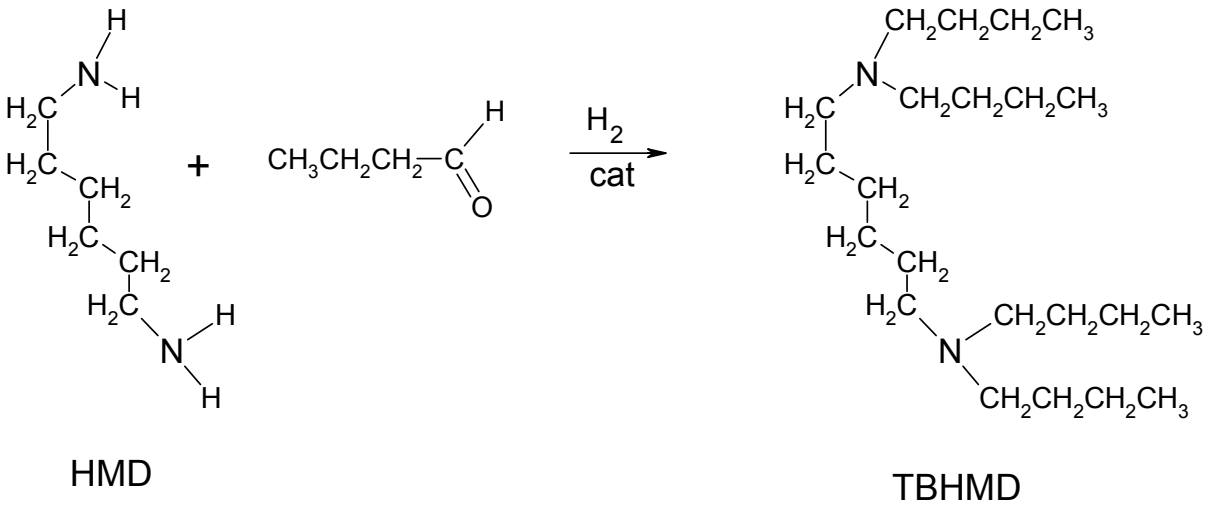
The chemical structure of TBHMD is shown below:



Tetrabutylhexamethylenediamine

Chemistry of Manufacture

TBHMD is made by the reductive addition of 4 equivalents of butyraldehyde to hexamethylene diamine in the presence of a catalyst, as shown below:



HMD TBHMD

A limited number of studies relevant to the EPA HPV-Program have been conducted on TBHMD. These studies are briefly reviewed in this testing rationale document. Robust summaries have been prepared for key studies using the IUCLID format. The available data set satisfactorily fulfills some the data requirements for the EPA HPV Program. As data from appropriate surrogate chemicals were not found, studies are proposed to fill the additional HPV-recommended endpoints.

Physicochemical, Fate and Aquatic Toxicity Information

Physicochemical Data

Physicochemical data for TBHMD are available from the manufacturer and by estimation methods.

Table 1: Physicochemical Properties of TBHMD	
Melting Point	< -18° C (2)
Boiling Point	ca. 380°C @ 1010 hPa (3) 83°C @ 2.9 hPa (2)
Vapor Pressure	< 0.015 hPa @ 25˚ C (3)
Partition Coefficient	Log $K_{o/w}$ = 4.56-7.59 (4)
Water Solubility	1200 mg/L @ 25° C (2)

These properties indicate that at ambient temperatures TBHMD is a low volatility liquid with limited water solubility in pure water. The EPIWIN estimated value of the partition coefficient is given as a range representing the free base form (7.59) and the diprotonated form (i.e two positive charges or $TBHMD^{++}$) and suggests that TBHMD will partition preferentially into fat; therefore, on the basis of only the octanol-water partition coefficient, TBHMD is considered to have potential for bioaccumulation. Actual bioaccumulation, however, is dependent upon pH, biodegradation and oxidative metabolism-depuration of TBHMD in organisms. As the pK_a of tertiary amines is typically in the range of 10 to 11, most of the TBHMD will be in the protonated forms at the near neutral pH levels typically found in the environment and absorption by aquatic organisms may be limited.

Recommendation: No additional physicochemical studies are recommended. The available data fill the HPV required data elements.

Environmental Fate and Pathways

TBHMD's photodegradation was estimated using version 1.90 of the Atmospheric Oxidation Program for Microsoft Windows (AOPWIN) that estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The estimated rate constant is used to calculate atmospheric half-lives for organic compounds based upon average atmospheric concentrations of hydroxyl radical. The program produced an estimated rate constant of 213 E-12 cm^3/molecule-sec. Using the default atmospheric hydroxyl radical concentration in APOWIN and the estimated rate constant for reaction of

TBHMD with hydroxyl radical, the estimated half-life of TBHMD vapor in air is estimated to be approximately 0.6 hours (see accompanying robust summary for full details).

Water stability has not been quantitatively determined for TBHMD. No specific data were found for water stability of TBHMD or similar tertiary amines in the literature. Quantitative stability determinations (e.g. OECD 111) are not considered necessary for compounds containing only non-hydrolysable groups. Under these conditions, the SIDS manual states that consideration should be given to using an estimation method. Although amines are potentially hydrolysable (5), an estimate for this particular compound can be obtained using chemical principles. Assuming the reaction products of hydrolysis are the secondary amine and butanol, the enthalpy of the reaction can be calculated using standard bond energies. Although the entropy of the reaction cannot be easily estimated, estimates of the enthalpy of reaction and considerations of the free energy of the hydrolysis transition state indicate that hydrolysis is highly unlikely under environmental conditions. The hydrolytic half-life can therefore be reliably estimated at greater than one year. (6)

Aerobic biodegradation studies were not found for TBHMD. As the alkyl chains in TBHMD are not branched, it can be predicted that TBHMD will be biodegradable under aerobic conditions. Based on the slow rate of aerobic biodegradation of hexamethylenediamine (inherently biodegradable) (7) and the additional branching at the nitrogen centers, it is anticipated that TBHMD will not be "readily biodegradable" by the OECD definition. This information, combined with the limited uses of this material and disposal of waste material going to a wastewater treatment plant, suggests that an "inherent biodegradability" test (e.g. OECD 302 series) would be a more appropriate and valuable as an initial assay than a test of ready biodegradability (e.g. OECD 301 series).

Theoretical Distribution (Fugacity) of TBHMD in the environment was estimated using the MacKay EQC level III model set to estimate distribution after 100% release to water, which is considered the most probable scenario regarding the potential release of this material. The EPIWIN model was allowed to estimate fate parameters, however, actual water solubility and melting point were used in the estimate. Regarding materials such as TBHMD that ionize under environmental conditions, a problem with the EQC Level 3 model is that it cannot adequately handle materials that have an equilibrium of forms or states (such as electronic charge) under typical environmental conditions. For this reason, the EQC level 3 model was also run on the singly and doubly charged species as these are considered more representative of TBHMD in the normal environmental pH range. Details of the parameters used are in the accompanying robust summary for environmental distribution. Results of this estimate are shown below in Table 2.

As the ionization of the two tertiary amines is pH dependent and a dynamic process, it is difficult to obtain accurate estimates for environmental distribution. To better answer the question of which form of TBHMD predominates at environmental pH levels, the pK_a for each species was estimated. Calculations indicate that the pK_a for the ionization of the first nitrogen is about 10.2 and for the second nitrogen 9.5 (8). These estimated values indicate that the predominant species for at typical environmental pH levels with by the doubly charged form ($TBHMD^{++}$) and the material will remain primarily in water after water release. If the pH of the local

environment is changed due to excessive amounts of TBHMD causing an increase in pH, sediment distribution may become important.

Environmental Compartment	Species Modeled		
	TBHMD	$TBHMD^{+}$	$TBHMD^{++}$
○ Air	< 0.01%	< 0.01%	< 0.01%
○ Water	10.5%	29.8%	88.5 %
○ Soil	< 0.01%	< 0.01%	< 0.01%
○ Sediment	89.5%	70.2%	11.5 %

Table 2. EQC Modeling of Environmental Distribution of TBHMD after Release to Water.

Recommendation: An aerobic biodegradation study according to the OECD 302 test guideline is recommended to complete the HPV information regarding fate.

Ecotoxicity

No experimental aquatic toxicity data are available for TBHMD. An estimate of the potential toxicity of TBHMD was developed using the EPA ECOSAR program and is given in Table 3. The estimate was conducted for each of the three ionized forms of TBHMD as each form has a different $K_{o/w}$ and the toxicity estimate using ECOSAR is a function of the $K_{o/w}$. EPA has acknowledged that the aquatic toxicity of aliphatic amines has a strong pH dependency (9). The un-ionized form of the amine generally displays a greater toxicity than the ionized forms. The rationale for this effect is that uncharged species more readily cross biological membranes than charged forms. As the pK_a of aliphatic amines is in the range of 9 to 11, they exist almost entirely in their charged form near neutral pH. On the other hand, as water solutions of aliphatic amines are basic, moderate to high concentrations of amines in weakly buffered water increase the pH and can theoretically exacerbate their aquatic toxicity. The estimates for the uncharged form of TBHMD also exceed the stated limitations of the aliphatic amines SAR model (limits: fish, $K_{o/w} < 6.0$; daphnids, $K_{o/w} < 5.0$; algae, $K_{o/w} < 7.0$) and are thus considered of lower reliability.

Another uncertainty surrounding the estimated for fish and invertebrates in the possibility that a specific mechanism of action may be operative since bi-tertiary amines, especially of this structural type, have been shown to act as blockers at nicotinic ganglia and neuromuscular junctions in mammals (10). It is thought that the di-cation (in this case represented by $TBHMD^{++}$) is the active form of bi-tertiary amines with nicotinic-blocking

action. As TBHMD is thought to be primarily in the di-cationic form at physiological pH, this type of activity is considered possible; thus, the neutral organics SAR model may be inadequate for certain organisms.

Table 3: Comparative Estimated Aquatic Toxicity of TBHMD forms

Species	ECOSAR Prediction		
	TBHMD	TBHMD^{+}	TBHMD^{++}
Fish, 96-hour LC_{50}	0.025 mg/L	0.23 mg/L	2.71 mg/L*
Daphnia, 48-hour EC_{50}	0.004 mg/L	0.029 mg/L	-**
Algae, 96-hour EC_{50}	0.04 mg/L	0.123 mg/L	-**

* Estimate for 14-day fish

** ECOSAR would not provide estimate

Recommendation: Estimation methods for acute fish and invertebrate toxicity, and algal growth inhibition data are not considered to be of sufficient reliability to fill these HPV data points. It is proposed that these HPV endpoints be filled with OECD 201, 202 and 203 studies. As the test material is considered stable in solution, the use of static conditions under near-neutral (pH adjusted for the particular test protocol) with analytical determination of actual concentration is proposed.

Health Effects

Acute Toxicity

Oral Exposure

A rat gavage study using six dose groups of rats of each sex has been conducted. An LD_{50} of 380 mg/kg (95% confidence interval 330-430 mg/kg) was determined for TBHMD in this study. Deaths occurred from several hours to six days after administration with most occurring in four days. Clinical observations included reduced appetite and activity for three to nine days in survivors and increasing weakness, collapse and death in animals that did not survive the 14-day observation period. Upon gross necropsy, decedents showed hemorrhagic areas of the lungs, liver discoloration and gastrointestinal inflammation. (11)

Dermal Exposure

A dermal acute toxicity study of undiluted TBHMD has been conducted using New Zealand albino rabbits and is describe in detail in the accompanying robust summary. The study used one rabbit at each of four dose levels from 251 to 1,000 mg/kg and the LD_{50} was reported as being greater than 398 mg/kg and less than 631 mg/kg. The two rabbits that died on study (631 and 1,000 mg/kg) both expired on day one of the test. No cause of death was identified. Clinical sings in surviving rabbits were limited to reduced appetite and activity for three or nine days after dosing. (11)

Recommendation: No additional acute toxicity studies are recommended. The available data fill the HPV required endpoints for acute toxicity. Although the available studies do not meet all requirements of current OECD guidelines, the data are consistent and the studies have been conducted by a scientifically defensible method. Conduct of additional studies would not add significantly to our understanding of this material's toxicity and it is recommended that no additional acute toxicity studies be conducted using this material.

Repeat Dose Toxicity

Two oral-gavage repeated dose (28-day and 13-week duration) studies have been conducted with TBHMD. For the purposes of the HPV program, the 13-week study has been selected for presentation and summarization and is identified as the critical repeated-dose study for the HPV program because of the longer duration and because of the limitations of the 28-day study, which was identified as a range-finding study for the 13-week test.

The 13-week corn-oil gavage study was conducted with Charles River CD rats approximately 6 weeks old at initiation of dosing. Groups of 15 animals of each sex were dosed daily by corn-oil gavage at dose levels of 0, 2, 5 or 20 mg/kg body weight. Detailed observations were conducted once weekly and individual body weights and food consumption values were recorded weekly. Clinical pathology tests were run on 10 randomly selected rats/sex/group at 13 weeks of study and all test and control animals were sacrificed and necropsied after 13-weeks

of treatment. Administration of test substance for 13 weeks was associated with pathological changes in the liver of 20 mg/kg-day rats of each sex. Females appeared to be more affected. At 5 mg/kg-day, females showed slight liver pathology but males were not affected. Decrease in body weight gain, increase in leukocyte count, and increases in serum enzymes indicative of an hepatotoxic effect were also seen at the 20 mg/kg-day dose level. Although effects at 5 mg/kg-day were minor, it is considered a LOAEL and 2 mg/kg-day is considered the NOAEL for this study. (12)

Recommendation: No additional repeated-dose studies are recommended. The available data fill the HPV required endpoint for repeated-dose toxicity.

Genetic Toxicity

The SIDS/HPV requirement for genetic toxicity screening is for two end-points: generally one test sensitive for point mutation and one sensitive for chromosomal aberrations. No studies of TBHMD were found for either endpoint. The closest analogs with data that were identified were tributylamine (negative in the bacterial reverse mutation test and the mouse micronucleus test) and hexamethylenediamine (negative in the bacterial reverse mutation test and an *in vivo* chromosome aberration test). Although both of these similar materials lacked genotoxic activity, neither was considered a close enough analog to use as a high confidence surrogate for TBHMD.

Recommendation: It is proposed that the SIDS requirement for genetic testing be met by conducting a bacterial reverse mutation assay in accord with OECD-471 and an *in vitro* mammalian chromosome aberration test in accord with OECD-473.

Reproductive Toxicity

No studies of the effect of TBHMD on reproduction were found. Reproductive organs of rats in the 13-week study were not affected, suggesting that TBHMD has no specific activity on reproduction. Although this limited information suggests lack of effect on reproductive function, it does not meet the HPV guidelines in the absence of additional data from a reproductive screening test or a developmental toxicity study.

Recommendation: A combined reproductive-developmental toxicity-screening test in recommended by the oral route using the OECG 421 testing guideline. If possible, dosing by gavage using a neutralized aqueous solution of test material is recommended to reduce gastric irritation and more closely mimic a repeated-dose exposure situation in man.

Developmental Toxicity

Developmental toxicity studies of TBHMD were not found. It can be argued that as the test material is charged at physiologic pH and probably highly protein bound, it will not cross the placenta in concentrations that will selectivity affect the conceptus; however, the possibility that metabolites will cross the placenta and selectively affect the conceptus cannot be excluded in the absence of experimental data.

Recommendation: A combined reproductive-developmental toxicity-screening test in recommended by the oral route using the OECG 421 testing guideline. If possible, dosing by gavage using a neutralized aqueous solution of test material is recommended to reduce gastric irritation and more closely mimic a repeated-dose exposure situation in man.

Conclusions

With regard to the parameters specified in the EPA HPV Challenge program, it is concluded that the available information fills all of the HPV-Program data recommendations except for information related to biodegradation, aquatic toxicity, genetic toxicity and reproductive/developmental toxicity. Studies to fill these parameters are proposed as follows:

Category	Endpoint	Test
Fate	Aerobic Biodegradation	OECD TG 302
Aquatic Toxicity	Algal Inhibition	OECD TG 201
	Daphnia Immobilization	OECD TG 202
	Fish, Acute Toxicity Test	OECD TG 203
Genotoxicity	Bacterial Reverse Mutation	OECD TG 471
	In vitro Mammalian Chromosome Aberration Test	OECD TG 473
Mammalian Toxicity	Reproduction/Developmental Toxicity Screening Test	OECD TG 421

Table 4. Proposed Testing for TBHMD

References

1 Chemical Information System (CIS) file Database File: SANSS [Chemical Nomenclature, Formulas, Structures] CAS Registry Number: 27090-63-7, Source of Information: TSCA Inventory, CIS Record ID: SA-00177616

2 Measured value. Solutia Material Safety Data Sheet #027090637 version of Aug 31, 1998.

3 Estimated using MPBPWIN v1.40 program found in EPIWIN 3.05

4 Estimated using KOWIN v1.66 program found in EPIWIN 3.05 Range represents free base and diprotonated forms.

5 Harris, J.C. in Lyman W, Reehl, W and Rosenblat, D.(1990) Handbook of Chemical Property Estimation Methods. American Chemical Society, Washington D.C.

6 Estimate conducted by Toxicology and Regulatory Affairs, December 2004. See robust summary for details.

7 IUCLID Dataset, Hexamethylenediamine (124-09-4), IUCLID 2000 European Chemicals Bureau 18-Feb-2000.

8 Values calculated by Toxicology and Regulatory Affairs using the online SPARC calculator at http://ibmlc2.chem.uga.edu/sparc. See Robust Summary for details of parameters.

9 See description of the aliphatic amines category in: Toxic Substances Control Act (TSCA) New Chemical Program Chemical Categories, October, 2002 (http://www.epa.gov/oppt/newchems/chemcat.htm).

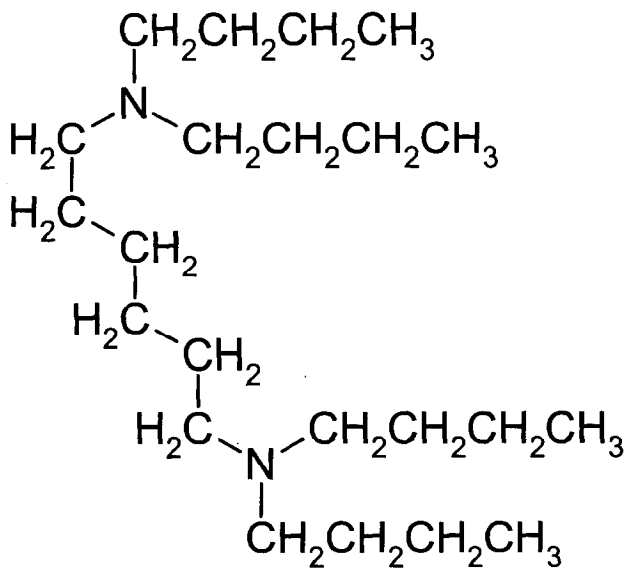
10 Hill, SA, RFP Scott and JJ Savarese, Structure-activity relationships: from tubocurarine to the present day. In: Goldhill and Flynn eds Muscle Relaxants. London: Baillere Tindall, 1994: 317-348.

11 Younger Laboratories Inc, Final Report: Acute Toxicity Testing of N,N,N',N' Tetrabutylhexamethylene diamine project YO-75-165, 07-29-1975; sponsored by Monsanto Co.

12 International Research and Development Corp., Final Report: Tetrahexamethylenediamine, 13-Week Oral Toxicity Study in Rats. Monsanto Study IR 83-153, Sponsored by Monsanto. April 18, 1985.

HPV Data Set

Tetrabutylhexamethylenediamine
CAS Number 27090-63-7



Existing Chemical	:	ID: 27090-63-7
CAS No.	:	27090-63-7
EINECS Name	:	N,N,N',N'-tetrabutylhexane-1,6-diamine
EC No.	:	248-219-2
Common name	:	TBHMD
Molecular Formula	:	$C_{22}H_{48}N_2$

Producer related part

Company	:	Solutia Inc, St. Louis MO
Creation date	:	06.01.2004

Substance related part

Company	:	Toxicology and Regulatory Affairs Freeburg IL, 62243 rauckman@toxicsolutions.com
Creation date	:	06.01.2004
Printing date	:	13.03.2005
Revision date	:	
Date of last update	:	10.03.2005
Number of pages	:	21

1.0.1 APPLICANT AND COMPANY INFORMATION

1.2 SYNONYMS AND TRADENAMES

1,6-Hexanediamine, N,N,N',N'-tetrabutyl- (8CI 9CI)

07.01.2004

N,N,N',N'-Tetrabutylhexamethylenediamine

07.01.2004

TBHMD

07.01.2004

Tetrabutylhexamethylenediamine

07.01.2004

2.1 MELTING POINT

Value	:	< -18 °C
Sublimation	:	
Method	:	
Year	:	
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Conducted by a standard method at the manufacturing plant.
Reliability	:	(2) valid with restrictions Data obtained by a scientifically defensible method.
Flag	:	Critical study for SIDS endpoint

07.01.2004 (8)

2.2 BOILING POINT

Value	:	= 83 °C at 2.9 hPa
Decomposition	:	
Method	:	
Year	:	
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Conducted by a standard method at the manufacturing plant.
Remark	:	The estimated boiling point at 1013 hPa from EPIWIN is: Boiling Pt (deg C): 380.18 (Adapted Stein & Brown method)
Reliability	:	(2) valid with restrictions Data obtained by a scientifically defensible method.
Flag	:	Critical study for SIDS endpoint

07.01.2004 (8)

2.3 DENSITY

Type	:	relative density
Value	:	= .82 at 24 °C
Method	:	
Year	:	
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4
Reliability	:	(2) valid with restrictions Data obtained by a scientifically defensible method.

07.01.2004 (8)

2.4 VAPOR PRESSURE

Value	:	ca. .000033 - .015 hPa at 25 °C
Decomposition	:	
Method	:	
Year	:	
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4

Remark :

VP: Using the MPVPBP program and correcting the EPIWIN estimate by the factor it under predicts the measured VP at 83 deg C (344), the corrected predicted VP at 25 deg C is 0.015 hPa based on a boiling point of 83 C at 2.2 mm Hg. This value has been added as the top of the VP range in the IUCILD entry. It is assumed that the measured vapor pressure is correct and this value for VP (0.015 hPa) is used in the fugacity calculations

Reliability : (2) valid with restrictions

Estimates made by an accepted method are assigned a reliability score of 2.

Flag : Critical study for SIDS endpoint

07.01.2004 (4)

2.5 PARTITION COEFFICIENT

Partition coefficient	:	octanol-water
Log pow	:	ca. 4.6 - 7.6 at 25 °C
pH value	:	ca. 9 - 11
Method	:	other (calculated)
Year	:	
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4

Method :

Octanol water partition coefficients for the three major forms of TBHMD were obtained through the KOWWIN program (v1.66) by entering the structure of the component into the program using the SMILES code. This program estimates the partition coefficient by summing the coefficients of all fragments of the molecule based on an empirical equation that has been validated.

As TBHMD is expected to exist in the free-base (unionized), the mono-protonated form and the di-protonated form in solution at nominal pH levels, the Ko/w was calculated for all three forms.

Result :

KOWWIN Program (v1.66) Results:
==========

Log Kow(version 1.66 estimate): 7.59 free base form
Log Kow(version 1.66 estimate): 6.08 N+ form
Log Kow(version 1.66 estimate): 4.56 N+N+ form

SMILES : CCCCN(CCCC)CCCCCCN(CCCC)(CCCC)
CHEM : TBHMD
MOL FOR: C22 H48 N2
MOL WT : 340.64

TYPE	NUM	LOGKOW FRAGMENT DESCRIPTION	COEFF	VALUE
Frag	4	-CH3 [aliphatic carbon]	0.5473	2.1892
Frag	18	-CH2- [aliphatic carbon]	0.4911	8.8398
Frag	2	-N< [aliphatic attach]	-1.8323	-3.6646
Const		Equation Constant		0.2290
			Log Kow =	7.5934

SMILES : CCCCN(CCCC)CCCCCCN(CCCC)(CCCC)(H)
CHEM : TBHMD-H+ (charged form)
MOL FOR: C22 H49 N2
MOL WT : 341.65

TYPE	NUM	LOGKOW FRAGMENT DESCRIPTION	COEFF	VALUE
Frag	4	-CH3 [aliphatic carbon]	0.5473	2.1892
Frag	18	-CH2- [aliphatic carbon]	0.4911	8.8398
Frag	1	-N< [aliphatic attach]	-1.8323	-1.8323
Frag	1	>N< [+5 valence; single bonds; H attach]	-4.6000	-4.6000
Factor	1	Reaction: nitrogen[+5] / polar group	1.2500	1.2500
Const		Equation Constant		0.2290
			Log Kow =	6.0757

SMILES : CCCCN(H)(CCCC)CCCCCCN(CCCC)(CCCC)(H)
CHEM : H-TBHMD-H++ (twice charged form)
MOL FOR: C22 H50 N2
MOL WT : 342.65
MANUAL CALCULATION EPIWIN WOULD ONLY ADD IN ONE CHARGED NITROGEN

TYPE	NUM	LOGKOW FRAGMENT DESCRIPTION	COEFF	VALUE
Frag	4	-CH3 [aliphatic carbon]	0.5473	2.1892
Frag	18	-CH2- [aliphatic carbon]	0.4911	8.8398
Frag	2	>N< [+5 valence; single bonds; H attach]	-4.6000	-9.2000
Factor	2	Reaction: nitrogen[+5] / polar group	1.2500	2.500
Const		Equation Constant		0.2290
			Log Kow =	4.5580

Conclusion :

Depending on the solution pH, the material will exist in solution in one of three forms ranging in Log Ko/w from 4.6 to 7.6. All three are expected to be present from about pH 9 to 11. Above or below this pH range, one form will predominate.

Reliability : (2) valid with restrictions

Estimates made by an accepted method are assigned a reliability score of 2.

Flag : Critical study for SIDS endpoint

07.01.2004 (7)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	= 1.2 g/l at °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	10.25 at 25 °C
Description	:	moderately soluble (100-1000 mg/L)
Stable	:	yes
Method	:	Conducted by a standard method at the manufacturing plant.
Remark	:	

Solubility of amines is a function of pH, this value is only accurate for solubility in pure water. This value can be confirmed by calculations based on pH and typical pKa values for tertiary amines and suggests that the material has a actual pKa of about 10.25.

The EPIWIN predicted solubility is of the material is only 0.05 g/L. On the other hand, the ionized form should be more soluble. If it is assumed that as material is dissolved, it is ionized until the pH of the solution increases to the pKa for the material, at which point the material in solution will be half ionized (the definition of the pKa). Then 0.12 g/L or 3.53 exp-4 M is in solution and 1.765 exp-4 M is ionized. This would put the hydrogen ion concentration at 5.67 exp-11 M, which corresponds to a pH for the solution of 10.25.

Although the pKa for TBHMD has not been measured, it is predicted to be 10.17 by the SPARC program, as this is very close to the calculated solution pH as the material approaches its reported solubility limit in pure water, it provides confirmation that the measured solubility of 0.12 grams per liter is a correct value for pure water.

The actual solubility under environmental conditions will be dependent on the starting pH of the water and its buffering capacity. Thus, under most environmental conditions, 0.12 g/L should be considered a lower limit on solubility.

Reliability	:	(2) valid with restrictions

Data obtained by a scientifically defensible method.

Flag	:	Critical study for SIDS endpoint

07.01.2004 (5) (8)

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	1500000 molecule/cm³
Rate constant	:	ca. .000000000213 cm³/(molecule*sec)
Degradation	:	ca. 50 % after .6 hour(s)
Deg. product	:	
Method	:	
Year	:	
GLP	:	
Test substance	:	as prescribed by 1.1 - 1.4

Method :

The structure was initially examined to determine if there was a chromophore that could absorb light energy at wavelengths above 295 um. As there is not, it was assumed that direct photolysis would be unimportant to the fate of the test material.

The APOWIN program was also run to determine an estimated rate of reaction with hydroxyl radical. This rate was used to estimate the half-life of TBHMD in the troposphere assuming a tropospheric hydroxyl radical concentration of 1,500,000 molecules hydroxy radical per cm3.

Result :

The calculated half-life is 0.6 hours based on 1,500,000 molecules of hydroxyl radical per cc.

```
AOP Program (v1.90) Results:
============================
SMILES : CCCCN(CCCC)CCCCCCN(CCCC)(CCCC)
CHEM   : TBHMD
MOL FOR: C22 H48 N2
MOL WT : 340.64

----- SUMMARY (AOP v1.90): HYDROXYL RADICALS---------
Hydrogen Abstraction       =  80.6730 E-12 cm3/molecule-sec
Reaction with N, S and -OH = 132.0000 E-12 cm3/molecule-sec
Addition to Triple Bonds   =   0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds =   0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings =   0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings    =   0.0000 E-12 cm3/molecule-sec

   OVERALL OH Rate Constant = 212.6729 E-12 cm3/molecule-sec
   HALF-LIFE =     0.050 Days (12-hr day; 1.5E6 OH/cm3)
   HALF-LIFE =     0.604 Hrs
```

Conclusion :

A value of approximately 0.6 hours is accepted as the atmospheric half-life of TBHMD in the troposphere due to indirect photolysis. No direct photolysis or reaction with atmospheric ozone is anticipated.

Reliability	:	(2) valid with restrictions Estimates made by an accepted method are assigned a reliability score of 2.
Flag	:	Critical study for SIDS endpoint

31.10.2004 (1)

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	at °C
t1/2 pH7	:	at °C
t1/2 pH9	:	at °C
Degradation	:	< 50 % after 1 year at pH and °C
Deg. product	:	
Method	:	
Year	:	
GLP	:	
Test substance	:	other TS

Method :

The stability of this material in water is estimated based on established chemical principles.

Result :

Although amines are potentially susceptible to hydrolysis*, experience suggests that these simple tertiary amines are resistant to hydrolysis. The only plausible mechanism for hydrolysis is protonation of the amine to the nitrogen-centered cation followed by Sn1 elimination of a carbocation with the charge residing on a primary carbon. As primary carbocations are poor leaving groups, this reaction is considered unlikely under normal environmental conditions.

The presence of a second tertiary amine center is not expected to influence the water stability of the compound as it is situated several carbons away.

Support for the hydrolytic stability of TBHMD also comes from thermodynamic considerations. The enthalpy of reaction for hydrolysis of a tertiary amine to a secondary amine and butyl alcohol is calculated by summing the strengths of bonds broken and subtracting the sum of the strengths of the bond formed. (Organic Chemistry by Peter Vollhardt, W.H. Freeman & Co, NY, NY 1987 pp71-73)

Bonds broken	
Water O-H	497 kJ
N-C	350 kJ

Bonds formed	
Alcohol C-OH	-356 kJ
Amine N-H	-382 kJ

Total estimated enthalpy of reaction = +109 kJ/mole

As the enthalpy of reaction indicates a significantly endothermic reaction and the transition state for hydrolytic reaction (primary butyl cation) is

relatively high energy (as compared to a tert-butyl cation, for example), this hydrolysis reaction is considered unlikely under environmental conditions.

Bond energies from Lide, Handbook of Chemistry 84th edition 2003-2004 section 9

* The aliphatic amine moiety is considered potentially susceptible to hydrolysis by Harris (J.C. Harris in Lyman W, Reehl, W and Rosenblat, D. Handbook of Chemical Property Estimation Methods. American Chemical Society, Washington D.C. 1990, page 7-6).

Test substance : Tetrabutylhexamethylenediamine (TBHMD) CASNO 27090-63-7

Conclusion :

Experiences with tertiary amines with similar structures indicate hydrolytic stability. Thermodynamic calculations of the enthalpy of hydrolysis also indicate that hydrolysis is an endothermic reaction. As the transition state is also considered to have a high delta G, hydrolysis of TBHMD is considered highly unlikely under environmental conditions. It can be concluded that TBHMD stable in water and has a hydrolysis half-life of greater than 1 year.

Reliability : (2) valid with restrictions

Estimates made by an accepted method are assigned a reliability score of 2.

Flag : Critical study for SIDS endpoint

09.03.2005 (2)

3.3.2 DISTRIBUTION

Media : other: air soil sediment and water

Method : Calculation according Mackay, Level III

Year :

Method :

Measured values for physical properties of TBHMD were input into EPIWIN as shown below. Default biodegradation rates were determined to be reasonable. Model was set to an initial distribution of 100% to water due to the material's low volatility and use pattern. The EQC Level 3 model (as found in EPIWIN 3.05) was utilized.

This material is an amino compound and the EC Level 3 model will not adequately handle the equilibrium states between the charged (protonated) forms and the uncharged form of the material. Because of this, the distribution was independently calculated for each form realizing that the actual distribution will be a pH dependent composite of these three calculations.

Result :

```
Level III Fugacity Model (Full-Output):
=======================================
  Chem Name   : TBHMD
  Molecular Wt: 340.64
  Henry's LC  : 5.6e-005 atm-m3/mole (calc VP/Wsol)
  Vapor Press : 0.015 mm Hg  (user-entered)
  Log Kow     : 7.59  (Kowwin program)
  Soil Koc    : 1.6e+007  (calc by model)
```

	Concentration (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.000311	1.21	0
Water	10.5	360	1000
Soil	1.01e-005	360	0
Sediment	89.5	1.44e+003	0

	Fugacity (atm)	Reaction (kg/hr)	Advection (kg/hr)	Reaction (percent)	Advection (percent)
Air	2.95e-015	2.36	0.0411	0.236	0.00411
Water	4.24e-012	266	138	26.6	13.8
Soil	3.19e-021	0.000258	0	2.58e-005	0
Sediment	1.27e-012	569	23.6	56.9	2.36

Persistence Time: 1.32e+003 hr
Reaction Time: 1.58e+003 hr
Advection Time: 8.15e+003 hr
Percent Reacted: 83.8
Percent Advected: 16.2

Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin):
Air: 1.207
Water: 360
Soil: 360
Sediment: 1440
Biowin estimate: 3.130 (weeks)

Advection Times (hr):
Air: 100
Water: 1000
Sediment: 5e+004

Level III Fugacity Model (Full-Output):

Chem Name : TBHMD-H+ (charged form)
Molecular Wt: 341.65
Henry's LC : 1.41e-013 atm-m3/mole (Henrywin program)
Vapor Press : 4.54e-009 mm Hg (Mpbpwin program)
Log Kow : 6.08 (Kowwin program)
Soil Koc : 4.93e+005 (calc by model)

	Concentration (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	2.73e-013	1.75	0
Water	29.8	208	1000
Soil	6.42e-009	208	0
Sediment	70.2	832	0

	Fugacity (atm)	Reaction (kg/hr)	Advection (kg/hr)	Reaction (percent)	Advection (percent)
Air	5.19e-027	5.73e-010	1.45e-011	5.73e-011	1.45e-012
Water	1.81e-019	525	158	52.5	15.8
Soil	6.59e-032	1.13e-007	0	1.13e-008	0
Sediment	3.24e-020	310	7.44	31	0.744

```
Persistence Time: 529 hr
Reaction Time:    634 hr
Advection Time:   3.21e+003 hr
Percent Reacted:  83.5
Percent Advected: 16.5

Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin):
   Air:      1.75
   Water:    208.1
   Soil:     208.1
   Sediment: 832.3
     Biowin estimate: 3.383  (days-weeks)

Advection Times (hr):
   Air:      100
   Water:    1000
   Sediment: 5e+004



=====================================
 Chem Name   : H-TBHMD-H++  (twice charged form)
 Molecular Wt: 342.66
 Henry's LC  : 8.91e-015 atm-m3/mole (Henrywin program)
 Vapor Press : 5.29e-013 mm Hg  (Mpbpwin program)
 Log Kow     : 4.56  (user-entered)
 Soil Koc    : 1.49e+004  (calc by model)

        Concentration   Half-Life    Emissions
          (percent)        (hr)       (kg/hr)
 Air       1.88e-011       3.18         0
 Water     88.5            208          1000
 Soil      9.72e-010       208          0
 Sediment  11.5            832          0

           Fugacity    Reaction    Advection   Reaction    Advection
            (atm)      (kg/hr)      (kg/hr)    (percent)   (percent)
 Air       2.01e-029    1.04e-008   4.79e-010   1.04e-009   4.79e-011
 Water     2.86e-020    750         225         75          22.5
 Soil      9.98e-033    8.23e-009   0           8.23e-010   0
 Sediment  5.29e-021    24.3        0.583       2.43        0.0583

 Persistence Time: 254 hr
 Reaction Time:    329 hr
 Advection Time:   1.13e+003 hr
 Percent Reacted:  77.4
 Percent Advected: 22.6

 Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin):
    Air:      3.182
    Water:    208.1
    Soil:     208.1
    Sediment: 832.3
      Biowin estimate: 3.635  (days-weeks)

 Advection Times (hr):
    Air:      100
    Water:    1000
    Sediment: 5e+004
```

Conclusion :

If released into water, regardless of the charged form, the amount distributing to air and soil will be negligible. Depending on the prevailing pH, the material will distribute into water and sediment with water being favored at lower pH levels and sediment at higher pH values.

Reliability : (2) valid with restrictions

Estimates made by an accepted method are assigned a reliability score of 2.

Flag : Critical study for SIDS endpoint
08.01.2004 (6)

3.5 BIODEGRADATION

4.1 ACUTE/PROLONGED TOXICITY TO FISH

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	= 380 mg/kg bw
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	
Vehicle	:	other: Corn oil
Doses	:	
Method	:	
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Method :

Groups of five young adult Sprague-Dawley rats (2 or 3 of each sex) were administered test material by intragastric intubation as a 25% solution in corn oil. At dosing, average group weight of males was 205 to 220 g and average group weight of females was 210-235 grams. Surviving animals were observed for 14 days and were sacrificed and necropsied.

Result :

Dose levels and results are given in the table

MALES and FEMALES

Dose	Mortality		
mg/kg	Males	Females	Combined
251	0/2	0/3	0/5
316	1/3	0/2	1/5
398	2/2	1/3	3/5
501	2/3	1/2	3/5
631	1/2	3/3	4/5
794	3/3	2/2	5/5

CLINICAL EFFECTS:
Mortality occurred from several hours to 6 days after dosing with most in four days

@ Lethal Doses: increasing weakness, collapse and death.

@ Nonlethal Doses: reduced appetite and activity for 3 to 9 days.

GROSS NECROPSY FINDINGS

Decedents: Hemorrhagic areas of lungs, liver discoloration and gastrointestinal inflammation.

Survivors: Lung congestion, viscera appeared normal at sacrifice.

Conclusion :

TBHMD has an acute oral LD50 in Sprague-Dawley rats of 380 mg/kg (95% CI 330 - 430). Males and females are approximately equally sensitive.

Reliability : (2) valid with restrictions

Study protocol was comparable to current OECD guideline, study not conducted under GLP.

Flag : Critical study for SIDS endpoint

01.11.2004 (9)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD50
Value	:	ca. 480 mg/kg bw
Species	:	rabbit
Strain	:	New Zealand white
Sex	:	male/female
Number of animals	:	4
Vehicle	:	other: none
Doses	:	
Method	:	
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4

Method :

One New Zealand albino rabbit of alternating sex per group was dermally exposed to undiluted test material at 4 dose levels. The test material remained in contact with the skin for 24 hours and was then removed. Surviving animals were observed for 14 days then were sacrificed and necropsied.

Result :

Dose levels and mortality results are given in the table

MALES and FEMALES

Dose mg/kg	Sex	Mortality	Time of death
251	F	0/1	
398	M	0/1	
631	F	1/1	one day
1000	M	1/1	one day

CLINICAL EFFECTS:
@ Lethal Doses: Rapidly increasing weakness, collapse and death.

@ Nonlethal Doses: reduced appetite and activity for 2 to 5 days.

GROSS NECROPSY FINDINGS

Decedents: Hemorrhagic areas of lung, liver discoloration, enlarged gall bladders, darkened spleen and gastrointestinal inflammation.

Survivors: Viscera appeared normal at sacrifice.

Conclusion	:	TBHMD is acutely toxic to rabbits by the dermal route with an acute dermal LD50 in rabbits between 398 and 631 mg/kg (geometric mean 480 mg/kg).
Reliability	:	(2) valid with restrictions Although the number of animals per group was small, sufficient data were generated by a scientifically defensible method to consider this a reliable estimate of dermal toxicity.
Flag	:	Critical study for SIDS endpoint

01.11.2004 (9)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-chronic
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	13 weeks
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	2, 5 or 20 mg/kg
Control group	:	yes, concurrent vehicle
NOAEL	:	= 2 mg/kg bw
LOAEL	:	= 5 mg/kg bw
Method	:	
Year	:	
GLP	:	yes
Test substance	:	

Method :

A 13-week corn-oil gavage study was conducted using Charles River CD rats(Charles River Breeding Laboratories, Inc., Portage, Michigan) approximately 6 weeks old at initiation. Groups of 15 animals of each sex were formed by randomly assigning animals using a computerized random selection in a block design based on body weights.

Animals were individually housed in wire-mesh cages in an environmentally controlled room. Fluorescent lighting provided illumination 12 hours per day. Water and diet were available ad libitum except during fasting for clinical pathology testing when food, but not water, was withheld. All animals were observed for overt signs of toxicity, moribundity and mortality twice daily. Detailed observations were conducted once weekly. Individual body weights and food consumption values recorded weekly. Test article was administered daily by corn-oil gavage at dose levels of 0, 2, 5 or 20 mg/kg body weight. Doses were adjusted weekly to the most recently obtained body weight.

CLINICAL PATHOLOGY: Laboratory tests were run on 10 randomly selected rats/sex/group at 13 weeks of study. The blood samples were obtained via puncture of the orbital sinus plexus from rats fasted overnight

(approximately 17 hours). Urine samples were collected during this 17-hour fasting period from rats housed individually in stainless steel metabolism cages

HEMATOLOGY PARAMETERS
Hematocrit value, hemoglobin concentration, erythrocyte count, MCH (calculated), MCV (calculated), MCHC (calculated), leukocyte count (total and differential), platelet count, reticulocyte count.

BIOCHEMISTRY PARAMETERS: Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, glucose, urea nitrogen, total bilirubin, cholesterol,
albumin, globulin (calculated), total protein, creatinine, electrolytes (sodium, potassium, chloride and calcium), phosphorus, ornithine carbamoyltransferase, gamma glutamyl transpeptidase, creatine phosphokinase.

URINALYSIS: Volume, color and appearance, pH, specific gravity, protein, glucose, ketones, urobilinogen, nitrites, bilirubin, occult blood, microscopy of spun deposit

NECROPSY: All animals were euthanized by carbon dioxide asphyxiation and received a complete post mortem examination under the direct supervision of a pathologist.

ORGAN WEIGHTS: Organs were weighed at terminal sacrifice only. Weights were recorded for liver, kidney (2), heart, adrenals (2), ovary(2),testis (2), brain.

HISTOPATHOLOGY: On all animals in the control and 20 mg/kg/day group sacrificed at study termination. Tissues fixed in formalin except eyes, which were fixed in a glutaraldehyde fixative, and stained with hematoxylin and eosin. All tissue masses and gross lesions were examined on all animals. Adrenal, liver, kidney and lung tissue of animals in the 2 and 5 mg/kg/day groups.

TISSUES PROCESSED: Adrenal (2), bone (femur), bone marrow (femur), bone marrow smear, brain (3 levels: fore, mid and hind brain), eye (2), gastrointestinal tract: esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum; gonads: ovary (2), testis with epididymis (2); heart, kidney (2), liver (2 sections), lung with mainstem bronchi (2), lymph nodes: mediastinal and mesenteric; mammary region (females only), pancreas, pituitary, prostate and seminal vesicle (2), salivary gland (mandibular with submandibular lymph node), sciatic nerve, skin, spinal cord (cervical, midthoracic and lumbar), spleen, thymic region, thyroid/ parathyroid complex, trachea, urinary bladder, uterus.

STATISTICS: Body weights (weeks 0-13), food consumption (weeks 1-13), clinical laboratory values (week 13) and organ weight (absolute and relative to body and brain weight, terminal sacrifice) data were analyzed using Bartlett's test for homogeneity of variance and analysis of variance (one-way classification). Treatment groups were compared to the control group, by sex, using the appropriate t-statistic (equal or unequal variance), as described by Steel, Torriel and Ostle. Dunnett's multiple comparison tables were used to determine significance. Total bilirubin, chloride, gamma glutamyl transpeptidase, ornithine carbamoyltransferase and specific

gravity were analyzed using a nonparametric approach, by transforming the data to ranks prior to analysis, as described by Conover and Iman. All statistical tests were two-tailed, with $p<0.05$ and $p<0.01$ used as levels of significance.

Result :

TEST SOLUTIONS: Test solutions analyzed during the study were within 10% of the target test article concentration. The mean concentration of all the analyzed solutions ranged from 98 to 105% of the desired levels. Solutions of tetrabutylhexamethylene diamine in corn oil stored for 24 hours or 9 days at room temperature were found to be stable.

SURVIVAL: All animals survived to the terminal sacrifice.

BODY WEIGHTS: Group mean body weights were statistically significantly lower than those of the vehicle control group for male rats in the 20 mg/kg-day dosage level group at week 1, and males and females in the 20 mg/kg-day dosage level group at weeks 2-13. There were also statistically significantly lower group mean body weight values for females in the 5 mg/kg-day dosage level group at weeks 12 and 13. There were no statistically significant differences in group mean body weights for males in the 2 and 5 mg/kg-day dosage level groups as compared to the male vehicle control group. With the exception of week 11, mean body weights in the 2 mg/kg-day females were not statistically different from the vehicle control mean weights.

BODY WEIGHTS AT TERMINATION (percent difference from control)

DOSE (mg/kg)	**males (g)**	**females (g)**
0	518	286
2	530 (+ 2.3)	272 (-4.9)
5	497 (- 4.0)	269* (-5.9)
20	338 *(-34.7)	205* (-28.3)

* p < 0.5

Clear test article related adverse effects were seen at the high-dose level (20 mg/kg/day). Severe weight gain depression and decreased food consumption were seen in both males and females. There were several other effects noted in the clinical pathology, organ weight and histopathology data which clearly indicated that the target organ of toxicity was the liver. At week-13, males had elevated alanine and aspartate aminotransferases and females showed elevated alanine and aspartate aminotransferases, alkaline phosphatase and cholesterol. Liver weights relative to body weights were elevated in animals of each sex (statistically significant only in females) and microscopic examinations indicated definite liver toxicity including cellular hypertrophy (6/15 males, 15/15 females) and toxic hepatitis (2/15 males, 15/15 females). Lesions characterizing the toxic hepatitis included multifocal inflammatory cell infiltration within lobules and portal triads, hepatocyte degeneration including cytoplasmic vacuolation and necrosis, increased mitosis and bile duct proliferation.

The same microscopic findings seen in the high-does group were seen in a few females in the 5 mg/kg-day group (hypertrophy 3/15 and toxic hepatitis 2/15). These findings were less severe then in the high-dose females and there were no correlative changes in serum biochemistry. In addition, females in the 5 mg/kg/day group had decreased body weights at week 13

(5.9% lower than the control mean) although this was not as severe as in the 20 mg/kg/-ay group females (28.3% lower than controls).

Both males and females in the 20 mg/kg/day groups also had elevated adrenal weights relative to body weights and microscopic evidence of trace to mild hypertrophy of cortical calls (8/15 males, 7/15 females). This was considered stress related and not a direct effect of the test article. Several other organ weight differences were noted, but there were no histopathological changes noted in any of these tissues. Therefore, these changes were likely a result of the body weight differences between the high dose and control animals.

GROSS EXAMINATION: No test article related macroscopic changes were observed among any of the terminally sacrificed male or female rats from the treatment groups.

LIVER HISTOPATHOLOGY RESULTS: Test article related microscopic changes were observed in the liver of male rats from the high dosage group and among female rats from the high and mid dosage groups. The liver changes consisted of toxic hepatitis and hepatocellular hypertrophy. The table shows the severity gradings of these changes by sex and group:

	MALES				FEMALES			
Dosage Level	0	2	5	20	0	2	5	20
Liver								
No. examined	15	15	15	15	15	15	15	15
Hepatitis, toxic,				(2)			(2)	(15)
Trace				2			2	2
mild								11
moderate								2
Hypertrophy, hepatocellular				(6)			(3)	(15)
Trace				6			3	2
Mild								11
Moderate								2

() = total number with lesion

The incidence of liver microscopic changes was minimal in the male rats in which only the high dosage group was affected. They were more pronounced in the females, in which both the high and mid dosage groups were affected.

TOXIC HEPATITIS was defined as multifocal inflammatory cell infiltration within lobules and portal triads, hepatocyte degeneration which included cytoplasmic vacuolation and necrosis (single cell or groups of cells), increased mitosis of hepatocytes and bile duct proliferation. All or some of these changes occurred in individual cases, depending upon the severity.

HEPATOCELLULAR HYPERTROPHY was defined as an increase in size of hepatic cells due to an increase in the size of the cytoplasmic compartment.

In addition to the hepatic lesions, trace to mild adrenal cortical cell hypertrophy was observed in high-dose male and female rats due to increased cytoplasmic accumulation of lipids. This was considered by the pathologist as a physiologic response resulting from non-specific stress and not directly related to the test article.

HEMATOLOGY: No test article related hematological changes were observed in the 2 and 5 mg/kg-day groups at termination. In the 20 mg/kg-day group, elevated leukocytes, characterized by elevations in both segmented neutrophils and lymphocytes were seen in animals of each sex. Other parameters were not affected, and in a few instances where statistical significance was seen, the differences were not considered to be of any biological significance.

BIOCHEMISTRY: No test article related biochemical changes occurred in the 2 or 5 mg/kg-day groups. Several biochemical parameters were affected in the 20 mg/kg-day group. Alanine aminotransferase was elevated in both males and females; the elevations in females were more pronounced. Females also showed elevations in aspartate aminotransferase, alkaline phosphatase, cholesterol and ornithine carbamoyltransferase. These findings correlate with the microscopic findings of toxic hepatitis and hepatocellular hypertrophy in these animals, and in particular, with the increased incidence and severity of liver changes seen in the females. Males additionally had decreases in creatinine, total protein, albumin and glucose when compared to control values. Decreases in total protein glucose and albumin could have occurred from both liver disease and malnutrition. Decreases in creatinine are occasionally seen but an exact mechanism is unknown. Decreases seen in females included creatinine and albumin.

URINALYSIS: There were no test article related changes in urinalysis values in males and females in the 2 and 5 mg/kg-day groups and males in the 20 mg/kg/day groups at the 13-week interval. Females in the 20 mg/kg-day group had an increase in urinary volume and a decrease in specific gravity. The significance of these findings is unknown.

Test substance :

Tetrabutylhexamethylenediamine (TBHMD) CASNO 27090-63-7

Conclusion :

Oral administration of test substance for 13 weeks was associated with pathological changes in the liver of 20 mg/kg-day rats of each sex. Females appeared to be more affected. At 5 mg/kg-day, females showed slight liver pathology but males were not affected. Decrease in body weight gain, increase in leukocyte count, and increases in serum enzymes indicative of an hepatotoxic effect were also seen at 20 mg/kg-day. Although effects at 5 mg/kg-day were minor, it is considered a LOAEL and 2 mg/kg-day is considered the NOAEL

Reliability : (1) valid without restriction

Guideline-like study conducted under GLPs with full documentation.

Flag : Critical study for SIDS endpoint

10.03.2005 (3)

5.5 GENETIC TOXICITY ‘IN VITRO‘

5.6 GENETIC TOXICITY ‘IN VIVO‘

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

(1) Calculated using EPIWIN 3.05 by Toxicology and Regulatory Affairs, October 2004

(2) Estimated by Toxicology and Regulatory Affairs based on accepted chemical principles. October 2004

(3) International Research and Development Corp., Final Report: Tetrahexamethylenediamine, 13-Week Oral Toxicity Study in Rats. Monsanto Study IR 83-153, Sponsored by Monsanto. April 18, 1985.

(4) EPIWIN 3.05, Syracuse Research Corporation 2000

(5) Calculation of solubility based on pKa by Toxicology and Regulatory Affairs, December 2003.

(6) Estimation made using EQC Model contained in EPIWIN 3.05 with additional inputs to accommodate the doubly charged form, by Toxicology and Regulatory Affairs, December 2003.

(7) Estimation made using KOWWIN Program (v1.66) with manual calculations to accommodate the doubly charged form, by Toxicology and Regulatory Affairs, December 2003.

(8) Solutia Material Safety Data Sheet #027090637 version of Aug 31, 1998.

(9) Younger Laboratories Inc, Final Report: Acute Toxicity Testing of N,N,N',N' Tetrabutylhexamethylene diamine project YO-75-165, 07-29-1975; sponsored by Monsanto Co.